Remarks

Claims 1-11 are pending in the subject application. Applicants have hereinabove amended claims 1, 2, 6 and 11. Applicants respectfully submit that amended claims 1, 2, 6 and 11 do not contain new matter and are fully supported by the specification as filed. Applicants respectfully request entry of this Amendment and reconsideration of the application as amended.

Claim 1 has been amended to include the recited definition of Z from claim 2. Support for the amendment to claim 1 can be found for example in claim 2; on page 3, lines 26-28, referring to wherein Z in formula 1 can be a pyridine or a thiophene group; and on page 4, lines 5-7, referring to wherein Z in formula 2 can be a pyridine or a thiophene group. Claims 2 and 6 have been amended to delete that part of the limitation that is included in claim 1. Claims 2 and 6 both depend from claim 1. Support for the amendment to claim 11 can be found for example on pages 21-24, referring to pharmaceutical compositions.

1. Rejection under 35 U.S.C. §102(b)

On page 2 of the Action, claims 1, 3-5, 7 and 9-11 stand rejected under 35 U.S.C. 102(b) as being anticipated by Venet (US Patent #5,968,952). The Examiner asserts that the teaching of Venet in example 83 on column 33 is encompassed by the instant claims. The Examiner asserts Venet's equivalent to Z to be benzodioxol, which the Examiner further asserts is a 9-membered heterocyclic that is substituted with 1-4 R^3 groups wherein R^3 is hydrogen. The Examiner has indicated that claims limited to Z being pyridine or thiophene would be free of prior art. Applicants have amended claim 1 to recite that Z is a pyridine or a thiophene that is optionally substituted with 1 to 4 R^3 substituents. Support for the amendment to claim 1 can be found for example in claim 2; on page 3, lines 26-28, referring to wherein Z in formula 1 can be pyridine or a thiophene group; and on page 4, lines 5-7, referring to wherein Z in formula 2 can be a pyridine or a thiophene group. The present amendment to claim 1 is believed to overcome the present rejection. Accordingly, Applicants respectfully request the Examiner to reconsider and withdraw his rejection under 35 U.S.C. 102(b)

2. Rejection under 35 U.S.C. §112, Second Paragraph

On page 3 of the Action, Claims 1, 2, 6 and 8 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants' amendments and/or arguments below are believed to overcome the present rejection.

With regard to claim 1, the Examiner asserts that this claim is ambiguous due to its reciting (on 13th and 14th line of this claim) "except H but including any optional fused rings referred to above". Applicants respectfully disagree with the Examiner's rejection of the aforementioned phrase. The claim is not ambigious; rather the inclusion of the phrase leave no ambiguity that the optional subsitutent R^3

may be a subsituent on each of the recited R^1 groups C_1-C_{10} alkyl, -$(CR^{11}R^{12})_qC(O)R^{10}$, -$(CR^{11}R^{12})_qC(O)OR^9$, -$(CR^{11}R^{12})_qOR^{10}$, -$(CR^{11}R^{12})_qC(R^{11})(R^{12})SO_2R^9$, -$(CR^{11}R^{12})_t$($C_3$-$C_{10}$ cycloalkyl), -$(CR^{11}R^{12})_t$(C_6-C_{10} aryl), and -$(CR^{11}R^{12})_t$(4 to 10 membered heterocyclic) **and on any of the optionally fused rings** to the recited R^1 cycloalkyl, aryl and heterocyclic. The inclusion of the rejected phrase makes it crystal clear where substituent R^3 may be placed. Accordingly, applicants respectfully submit that the phrase provides definiteness to the claim, not indefiniteness. Applicants respectfully request the Examiner reconsider and withdraw his rejection. With regard to claims 2 and 6, the Examiner asserts that these claims are ambiguous owing to the recitation (in the second line of this claim) "Z is a pydridine or thiophene group, including pyridine or thiophene groups substituted with . . .". The Examiner has suggested that this phrase should be rewritten to state "Z is a pyridine or thiophene optionally substituted with 1-4 R^3 substituents. Applicants' amendment of claim 1 is believed to overcome this rejection, as claims 2 and 6 depend from claim 1.

With regard to claim 8, the Examiner asserts that this claim is ambiguous due to the claim being drawn to a compound but having the word "and" on line 11 of the claim. The Examiner asserts that this leads to the claim being a composition and suggests that the claim be rewritten to have "or" in place of "and". Applicants respectfully disagree with the position taken by the Examiner and traverse this rejection of claim 8. Applicants respectfully point out that Markush language is employed in claim 8, and the use of the term "and" between the penultimate and ultimate groups of said Markush group is proper. Thus claim 8 does not refer to a composition as suggested by the Examiner but to a **compound** selected from the **group consisting of** the listed compounds **and** the pharmaceutically acceptable salts, solvates and prodrugs of the foregoing compounds as recited in this claim. It is respectfully submitted that such recitation represents the correct and proper use of Markush language. Accordingly, Applicants respectfully request the Examiner to withdraw her rejection of this claim.

3. Rejection under 35 U.S.C. §112, First Paragraph Written Description

On pages 3-4 of the Action, claims 10-11 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner alleges that these claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner asserts that the instant specification does not adequately describe the nexus between the inhibition of the Ras farnesylation receptor and a useful treatment of abnormal cell growth and the treatment of cancers.

Applicants respectfully disagree with the position taken by the Examiner and traverse this rejection.

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed. *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976)

("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims"). MPEP 2163.

An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). Under *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991), to satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed. The test for sufficiency of support in a parent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting *In re Kaslow*, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)). MPEP 2163.02.

It is respectfully submitted that the Examiner's assertion that the instant specification does not adequately describe the nexus between the inhibition of the Ras farnesylation receptor and a useful treatment of cancer is not well founded. Applicants state (on page 1, lines 20-26 of the instant application):

> Mutated, oncogenic forms of Ras are frequently found in many human cancers, most notably in more than 50% of colon and pancreatic carcinomas (Kohl et al., Science, Vol. 260, 1834 to 1837, 1993). The compounds of the present invention exhibit activity as inhibitors of the enzyme farnesyl protein transferase and are therefore believed to be useful as anti-cancer and anti-tumor agents.

Applicants have therefore provided reasonable basis for their assertion that inhibition of farensyl protein transferase is linked to the treatment of cancer. Furthermore, prior to the filing of the present application, it has been well known to those of ordinary skill in the art, that farnesyl transferase inhibitors can be useful as anticancer agent. Applicants respectfully direct the Examiner's attention to International patent publication WO 97/16443 (published May 9, 1997) which published prior to the filing of the present application and which has been cited as a reference by the Examiner in the instant case. On page 1, lines 9-31 of WO 97/16443, it is stated:

> Oncogenes frequently encode protein components of signal transduction pathways which lead to stimulation of cell growth and mitogenesis.

> Oncogene expression in cultured cells leads to cellular transformation, characterized by the ability of cells to grow in soft agar and the growth of cells as dense foci lacking the contact inhibition exhibited by non-transformed cells. Mutation and/or overexpression of certain oncogenes is frequently associated with human cancer. A particular group of oncogenes is known as ras which have been identified in mammals, birds, insects, mollusks, plants, fungi and yeasts. The family of mammalian ras oncogenes consists of three major members ("isoforms"): H-ras, K-ras and N-ras oncogenes. These ras oncogenes code for highly related proteins generically known as $p21^{ras}$. Once attached to plasma membranes, the mutant or oncogenic forms of $p21^{ras}$ will provide a signal for the transformation and uncontrolled growth of malignant tumor cells. To acquire this transforming potential, the precursor of the $p21^{ras}$ oncoprotein must undergo an enzymatically catalyzed farnesylation of the cysteine residue located in a carboxyl-terminal tetrapeptide. Therefore, inhibitors of the enzyme that catalyzes this modification, farnesyl protein transferase, will prevent the membrane attachment of $p21^{ras}$ and block the aberrant growth of ras-transformed tumors. Hence, it is generally accepted in the art that farnesyl transferase inhibitors can be very useful as anticancer agents for tumors in which ras contributes to transformation.
>
> Since mutated, oncogenic forms of ras are frequently found in many human cancers, most notably in more than 50% of colon and pancreatic carcinomas (Kohl et al., Science, vol 260, 1834-1837, 1993), it has been suggested that farnesyl tranferase inhibitors can be very useful against these types of cancer.

Applicants assert that the present specification in combination with the state of the art at the time of filing the present application clearly indicate that the Applicants were in possession of the invention as claimed in claims 10-11. Accordingly, Applicants respectfully request the Examiner to withdraw this rejection.

4. <u>Rejection under 35 U.S.C. §112, First Paragraph Enablement</u>

On pages 4-8 of the Action, claims 10-11 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing with comply with the enablement requirement. The Examiner alleges that these claims contain subject matter which was not described in the specification in such a way as to enable one skilled

in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner asserts that one of ordinary skill in the art would have to engage in undue experimentation to test which diseases can be treated by the compounds of the instant claims, with no assurance of success. For the reasons that follow, Applicants respectfully traverse this rejection and request that it be withdrawn.

Applicants respectfully submit that the Examiner has failed to make a *prima facie* case of non-enablement for the pending claims of the subject application under 35 U.S.C. §112, first paragraph that is well-grounded in scientific reasoning or evidence. The Examiner has failed to set forth a reasonable explanation as to why he believes that the scope of protection is not adequately enabled by the description of the invention provided in the specification. In order to make a §112, first paragraph rejection the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure).

The CCPA has stated that "it is incumbent upon the Patent Office, whenever a rejection on this basis [35 U.S.C. 112, first paragraph] is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." *In re Marzocchi*, 169 USPQ 368, 370 (CCPA 1971). Applicants respectfully submit that the Examiner has failed to meet his burden and has neither provided evidence or reasoning, which would support his assertion that the claims of the subject application are not enabled.

The Examiner has rejected the claims of the subject application by asserting that one of ordinary skill in the art would have to engage in undue experimentation to test which diseases can be treated by the compounds of the instant claims, with no assurance of success.

The specification clearly sets forth on page 1, that the compounds of the present application inhibit the enzyme farnesyl protein transferase and that such activity has been correlated with the treatment of cancers and tumors. Applicants reiterate their arguments set forth above under "written description" to support their position that it is well known that inhibition of farnesyl protein transferase will be useful in the treatment of abnormal cell growth and cancer. Applicants respectfully submit that a large number of compounds having tyrosine kinase inhibitory properties have been identified in the art. In this regard, applicants direct the Examiner's attention to the present application, page 1, line 27 to page 2, line 3, which lists patent applications describing compounds having activity inhibiting farnesyl protein transferase. Applicants are also attaching herewith a review article entitled Inhibitors in protein farnesylation 1998 in the journal Expert Opinion on Therapeutic Patents (1998) 8 (5), which is a review of different types of protein farnesyltransferase inhibitors prior to the filing of the present application.

Applicants respectfully submit that there is a high level of expertise in the field of the present invention. Applicants also respectfully submit that the specification of the claimed invention coupled with the high degree of expertise in the field of farnesyl transferase inhibitors enables one of ordinary skill in the art to practice the claimed invention.

The Examiner has offered no objective evidence for his position that the claimed invention is not enabled. The Examiner has merely offered his opinion without any documented support for such a position. The Examiner's position is contrary to the understanding of those of ordinary skill in art as indicated and shown by the multitude of patent applications direct to compound useful in the treatment of abnormal cell growth. The specification teaches that all the compounds employed in the pharmaceutical compositions and methods of these claims are inhibitors of the enzyme farnesyl protein transferase and that they are useful in treating abnormal cell growth including cancer. The Examiner has not proffered any evidence to show that those skilled in the art would doubt the objective truth of these statements. Therefore, such statements must, as indicated above, be accepted as true.

On pages 24-25 of the specification, Applicants provide further guidance for those skilled in the art to assess the activity of the compounds falling within the scope of the present invention using the recited *in vitro* and *in vivo* tests. Moreover, pages 22 and 23 of the specification describe how the methods of claimed invention can be carried out by those skilled in the art. It specifies, on these pages, appropriate dosages and methods of administration. This description includes the various modes by which the compounds employed in the claimed methods can be administered to mammals, the pharmaceutically acceptable forms in which they can be administered and appropriate dosages for their administration. The foregoing information is sufficient to enable one skilled in the art to practice the inventions of each of the pending claims and thus complies with the requirements of 35 U.S.C. §112, first paragraph.

A specification disclosure that contains a teaching of the manner and process of making and using the invention in terms that correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with enabling requirement of the first paragraph of §112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. *In re Marzocchi*, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971). Further, the burden is on the Examiner to come forth with evidence to establish a *prima facie* case of non-enablement. *In Re Armbruster*, 185 U.S.P.Q. 152, 153 (C.C.P.A. 1975); *In re Marzocchi*, 169 U.S.P.Q. at 370. The Examiner has not proffered any evidence, merely her opinion and a boilerplate list of factors for the lack of enablement of the claimed invention.

Applicants believe it is incontrovertible that those of ordinary skill in the art accepted that development of inhibitors of protein farnesyl transferase would be useful in the treatment of cancer as presently claimed. The Examiner has offered no objective evidence, which would contradict or question this understanding in the art at the time the filing of the subject application. Applicants respectfully

request that the Examiner reconsider and withdraw his rejection of claims 10-11 under 35 U.S.C. §112, first paragraph for the reasons set forth above.

With regard to claim 11, the Examiner has stated on page 8 of the Action, that she has included this claim in this rejection because the pharmaceutical composition is for use in the treatment of abnormal cell growth. She has indicated that a claim limited to the pharmaceutical composition of a compound of claim 1 and an acceptable pharmaceutical carrier would be in better condition for allowance. While Applicants do not concede that claim 11 is not allowable as originally written and have indeed traversed the present enablement rejection of this claim above, to advance prosecution, Applicants have amended this claim in the manner suggested by the Examiner. Accordingly, amended claim 11 is deemed to overcome this rejection.

On pages 8-13 of the Action, claims 1, 3-5, 7 and 9-11 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing with comply with the scope of enablement requirement. The Examiner asserts that Z is enabled by the instant specification to the extent that Z is optionally substituted pyridine or thiophene; however, all heterocyclic groups are not enabled. The Examiner has indicated that this rejection can be overcome by amending Z to be a pyridine or thiophene. Applicants' amendment of claim 1 (all other claims depend from claim 1) wherein Z has amended to be a pyridine or thiophene group is deemed to overcome this rejection.

CONCLUSION

Applicants respectfully request prompt consideration of the pending claims and early allowance of the application.

If the Examiner wishes to comment or discuss any aspect of this application or response, applicants' undersigned attorney invites the Examiner to call him at the telephone number provided below.

Respectfully submitted,

Date: July 29, 2004

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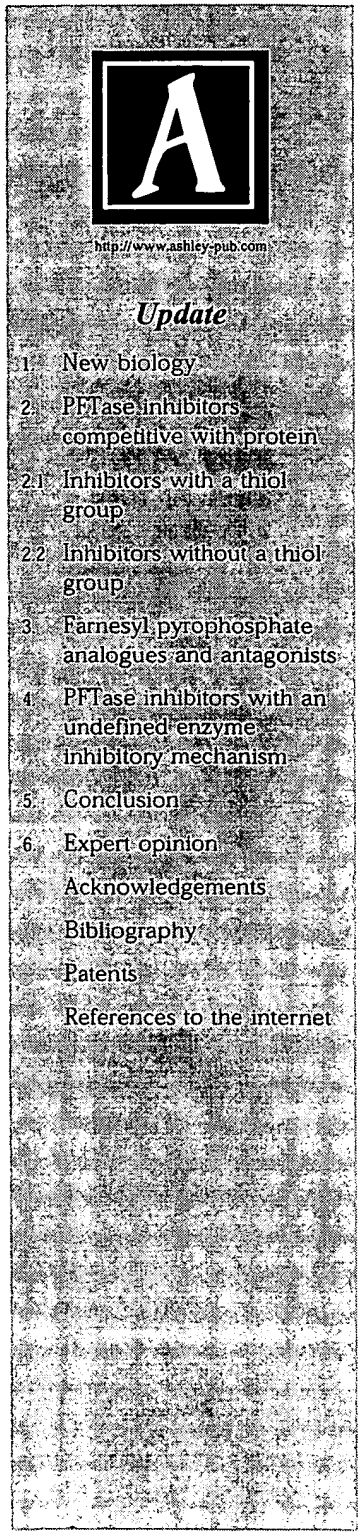
Expert Opinion on Therapeutic Patents



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Update

Inhibitors of protein farnesylation 1998

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Protein farnesyltransferase (PFTase) inhibitors are being studied as mechanistically novel antitumour agents. Whilst the antiproliferative effects of PFTase inhibitors are well-documented in cell culture and rodent animal models, clinical studies which began in 1997 should soon reveal their utility in human cancer patients. This review summarises the scientific and patent literature covering PFTase inhibition that has been published since the previous two updates [1,2]. New biology with a potential impact on the utility of PFTase inhibitors is reviewed first, followed by a discussion of new PFTase inhibitors. As in earlier updates, compounds are grouped according to their kinetic mechanism of PFTase inhibition.

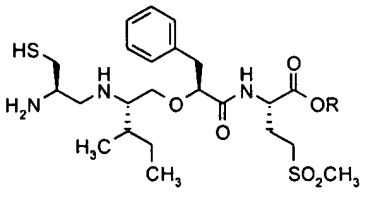
Keywords: *PFTase, protein farnesyltransferase, protein farnesyltransferase inhibitors, protein prenylation, ras oncogene*

Exp. Opin. Ther. Patents (1998) 8(5):553-569

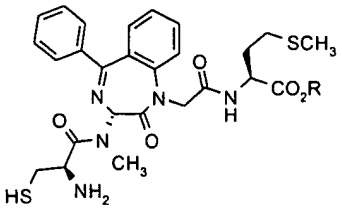
1. New biology

Interest in PFTase inhibitors began when it was determined that the protein product of the *ras* oncogene was a PFTase substrate [1]. Since then, studies in genetically engineered and human tumour cell lines with *ras* mutations have demonstrated that PFTase inhibitors suppress Ras farnesylation and function as antiproliferative agents [3]. The presence of a *ras* mutation is not a prerequisite for growth inhibition, since human tumour cell lines with either mutant or wild-type *ras* are sensitive to the antiproliferative effect of PFTase inhibitors [4,5]. It is possible that this added sensitivity is due in part to blocking prenylation of other (non-Ras) PFTase protein substrates involved with mitogenesis. *In vivo*, PFTase inhibitors suppress *ras*-dependent tumour growth in mice. Experiments in H-*ras* transgenic mice showed that **L-744,832** regressed tumours when administered subcutaneously once daily [6]. More recently, tumours in H-*ras* transgenic mice with an additional genetic defect, such as loss of the p53 tumour suppressor gene or deregulated *myc* transcriptional activation, regressed upon similar treatment with **L-744,832** [7]. The exact mechanism of tumour regression during PFTase inhibitor treatment has not been elucidated, although it may involve apoptosis [8]. Collectively, these results suggest a range of human tumours that might respond to PFTase inhibitor therapy. Synergy between PFTase inhibitors and other chemotherapeutic agents may further extend the therapeutic potential. Careful preclinical evaluation of drug combinations is warranted, since a study examining the effect of **BZA-5B** in combination with cisplatin unexpectedly found that **BZA-5B** actually induced cisplatin resistance in a melanoma cell line with an N-*ras* mutation [9].

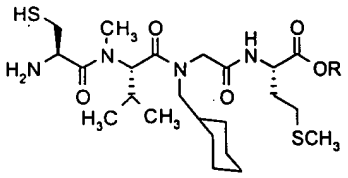
Cells with overexpressed H-*ras* were known to be much more sensitive to the effects of PFTase inhibitors than cells with overexpressed K-*ras*, which



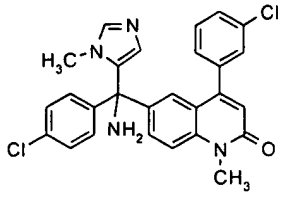
L-744,832 R = $CH(CH_3)_2$
L-739,749 R = CH_3
PFTase IC_{50} R = H 0.3 nM



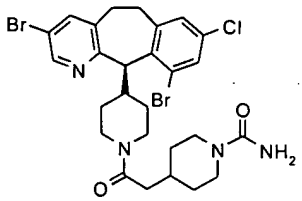
BZA-5B R = CH_3
PFTase IC_{50} R = H 0.3 nM



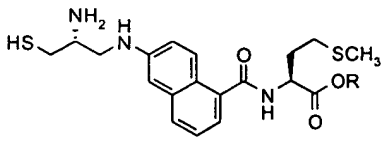
HR-11 R = H PFTase IC_{50} 1.2 nM
HR-12 R = CH_3



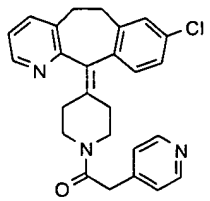
(+) isomer R115777
PFTase IC_{50} 2 nM



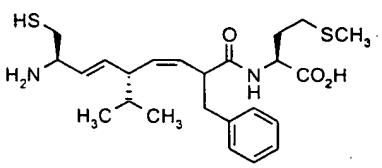
SCH 66336
PFTase IC_{50} 2 nM



RPR 113829 R = H PFTase IC_{50} 1.8 nM
RPR 114334 R = CH_3



SCH 44342
PFTase IC_{50} 250 nM



B956
PFTase IC50 11 nM

is the most prevalent *ras* mutation in human tumours. It was subsequently found that K-Ras is not only farnesylated by PFTase, but is also geranylgeranylated by protein geranylgeranyltransferase I (PGGTase I) *in vitro*. Since geranylgeranylated K-Ras is thought to retain some biological activity, the alternative prenylation pathway might be responsible for the decreased sensitivity to PFTase inhibitors in K-Ras transformed cells. Direct evidence for the geranylgeranylation of K-Ras in cells treated with PFTase inhibitors has been obtained. HPLC analysis showed that geranylgeranylated K-Ras is present in cells treated with **B956** [10] or **SCH 44342** [11] and [^{3}H]-mevalonolactone, whereas exclusively farnesylated K-Ras is present in untreated cells. Although the relationship between the overall prenylation status of H- or K-Ras and tumour growth needs further study, it is interesting to note that distinct biological functions for H-Ras and K-Ras may exist. The first evidence for this is a prenylation-dependent binding of K-Ras with microtubules which does not occur with H-Ras [12]. In addition to prenylation, an intact CaaX box was required for K-Ras microtubule binding. Association of prenylated K-Ras with the plasma membrane was disrupted in cells treated with Taxol (paclitaxel). This suggests either that microtubules are involved with delivering K-Ras to the plasma membrane, or that prenylated (but not fully processed) K-Ras has some unknown function related to microtubules.

Clinical trials with two PFTase inhibitors, **R115777** and **SCH 66336**, were initiated in 1997 by Janssen (in conjunction with the National Cancer Institute [201]) and Schering-Plough, respectively [13-15]. Preliminary results from these trials are scheduled to become public in 1998. In a related study, Isis Pharmaceuticals began clinical trials with an antisense oligodeoxynucleotide designed to block expression of the H-*ras* gene *in vivo* [16]. It will be interesting to compare the results of the *ras* antisense trial, designed to block the activity of one farnesylated protein, with results from

PFTase inhibitor clinical trials, which will affect the activity of multiple PFTase protein substrates.

A number of studies offer evidence that Ras is not the only PFTase substrate responsible for the antiproliferative effect of PFTase inhibitors in transformed cells [17,18]. While Ras is an integral part of the MAP kinase signalling pathway, RhoB, a small GTP-binding protein which is both farnesylated and geranylgeranylated, is part of a related signalling pathway involved with focal adhesion assembly and actin cytoskeleton organisation. The PFTase inhibitor **L-739,749** was recently shown to block the farnesylation of RhoB both *in vitro* and in intact cells, and to inhibit RhoB-dependent cell growth [19]. In this experiment, the level of geranylgeranylated RhoB increased as farnesylated RhoB decreased. Other PFTase substrates which may play a role in the antiproliferative effects of PFTase inhibitors include RhoE [20], RheB [21,22], and the protein tyrosine phosphatases PRL-1 [23,24], and IP3 5-PTPase [25].

An x-ray crystal structure of wild-type rat PFTase was determined at 2.25 Å resolution [26,27]. The structure, composed of α and β subunits, consists of a seven-helix hairpin domain and an α helical barrel structure thought to be the farnesylpyrophosphate (FPP) binding site. The catalytic zinc ion is positioned at the entrance to the α-α barrel, lending support to this interpretation. Mutational analysis of conserved residues in the β-subunit of PFTase confirm structural evidence that residues His^{248}, Arg^{291}, Lys^{294} and Trp^{303} are involved with binding FPP, and that Cys^{299}, Asp^{297} and His^{362} are ligands for the catalytic zinc cation [28]. Point mutation also determined that Arg^{202} is a critical residue for binding the C-terminal carboxylate of the protein substrate.

2. PFTase inhibitors competitive with protein

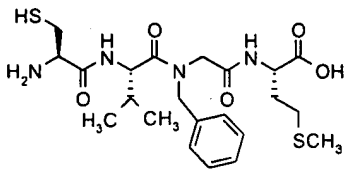
2.1 Inhibitors with a thiol group

The C-terminal tetrapeptide of Ras comprises a CaaX motif found in many proteins that are substrates for prenyltransferases (C = cysteine, a = any aliphatic amino acid, X = serine or methionine for PFTase substrates; X = leucine for PGGTase I substrates). Enzyme substrates undergo farnesylation of the cysteine thiol of the CaaX sequence. Early studies established that tetrapeptides corresponding to the C-terminus of the Ras protein were Ras-competitive inhibitors of PFTase. Much about the biological consequences of PFTase inhibition was first revealed by studying tetrapeptide and modified tetrapeptide inhibitors. Since the genesis and evolution of protein substrate-based inhibitors are the subject of earlier reviews, only the most recent extensions of this work are summarised below [3,29].

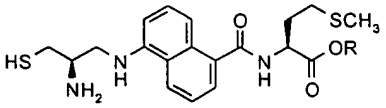
Transposing the hydrophobic sidechains in the tetrapeptide inhibitor Cys-Val-Phe-Met (CVFM) from C_α to N, investigators at the Hebrew University of Jerusalem report a series of highly potent PFTase inhibitors that are structurally related to compounds disclosed in a Merck patent application [30,101]. The N-benzyl glycine analogue **1** was only 2-fold less active than the parent CVFM. The most potent compound in the series, **HR-11** inhibited PFTase with an IC_{50} of 1.2 nM, while the methyl ester prodrug **HR-12** blocked farnesylation in v-H-*ras* transformed NIH-3T3 cells with an IC_{50} of 10 μM. As with the parent CVFM tetrapeptide, these compounds were not substrates of the enzyme and were highly selective for the inhibition of PFTase over PGGTase I.

In a scientific paper, researchers at Rhône-Poulenc Rorer discuss conformationally restricted napthalene-containing CaaX analogues, inhibitors previously disclosed in patent applications [31]. Both 1,5- (**2a**) and 1,6- (**RPR 113829**) substituted napthalenes fit the authors' pharmacophore model of an extended, biologically active conformation which is consistent with observations from the group formerly at the University of Pittsburgh [32-34]. These compounds inhibited PFTase with IC_{50} values of 5.6 nM (**2a**) and 1.8 nM (**RPR 113829**). As with many carboxylate PFTase inhibitors, the parent acids did not suppress farnesylation in cell culture, although the corresponding methyl ester prodrugs **2b** and **RPR 114334** showed 20 - 50% inhibition of Ras farnesylation in CCL39 cells transfected with activated H-*ras*. **RPR 114334** inhibited anchorage-independent cell growth in genetically engineered and human tumour cell lines, suppressing colony formation with IC_{50} values of 5 μM and 10 μM for H- and K-*ras* transfected NIH-3T3 cells, respectively, and 5 μM (H 460 cells) and 50 μM (HCT 116 cells) for two human tumour cell lines containing K-*ras* mutations. A Rhône-Poulenc Rorer patent application claims 1,4-substituted naphthalenes (**3**) as PFTase inhibitors [102].

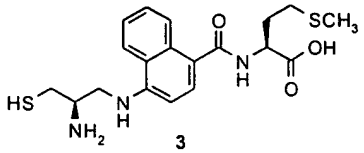
The same group also describes modifications to the N-terminus of Cys-Val-Tic-Met (**4**) intended to stabilise the tetrapeptide towards proteolytic



1
PFTase IC_{50} 100 nM



2a R = H PFTase IC_{50} 5.6 nM
2b R = CH_3



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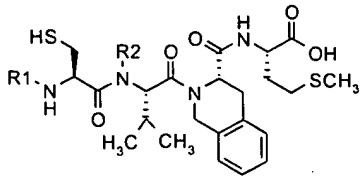
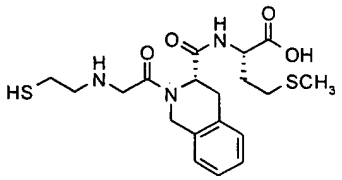
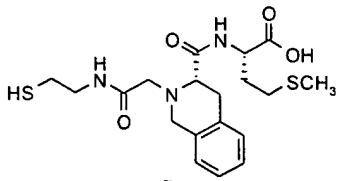


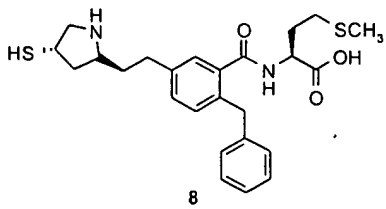
4 R1,R2 = H PFTase IC_{50} 10 nM
5 R1 = $CH_3(CH_2)_{10}CO$, R2 = H PFTase IC_{50} 213 nM



6
PFTase IC_{50} 100 nM



7
PFTase IC_{50} 2.1 µM



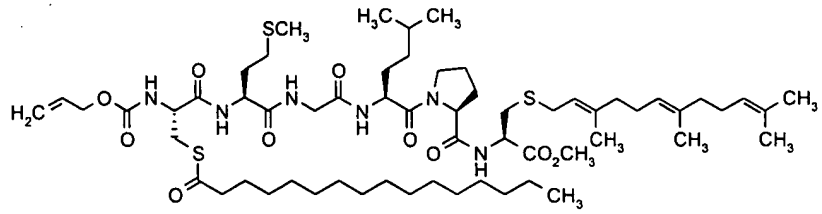
8

degradation [35]. N-Acylated tetrapeptides such as **5** had *in vitro* potencies similar to the parent tetrapeptide **4**, but were more stable to incubation with CCL39 cell extract. Despite the increase in stability, these compounds were still not active in cell culture, perhaps reflecting poor permeability of cell membranes. Ester prodrugs of **5** were not reported. Peptide backbone modifications, such as inverting the peptide attachment of **6** [36] to give **7** or reduction of the Tic-Met amide bond, were poorly tolerated in terms of *in vitro* potency [35].

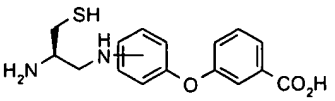
A patent application discloses that Zeneca combined the 2*(R)*-mercaptopyrrolidine cysteine replacement (also used as a constrained cysteine residue by Bristol-Myers Squibb [37] with a diphenylmethylcarboxamide $\alpha_1\alpha_2$ surrogate, giving **8** [103]. Biological data was not reported.

To examine the possibility of product feedback inhibition, investigators at the Universität Karlsruhe synthesised *(S)*-farnesylated peptides corresponding to the C-terminal tri- and pentapeptide sequence of the fully processed N-Ras protein [38]. While not inhibiting PFTase up to millimolar concentrations, extension to the farnesylated and palmitoylated hexapeptide **9** revealed weak inhibition.

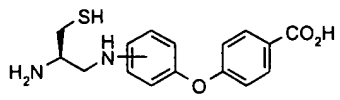
Non-peptide, diarylether PFTase inhibitors comprise the subject of a recent communication from Merck [39]. Structurally related to biarylcarboxylate inhibitors published earlier [40,41], diarylether carboxylates **10a,b** and **11a,b** were moderately active *in vitro*, and demonstrated a preference for the *m,m*-substitution pattern. The methyl ester of **10b** blocked the anchorage-independent growth of H-*ras* transformed cells in soft agar with an IC_{50} of 10 - 30 µM. Replacing the carboxylate group of **10a** with a methyl group did not substantially change the IC_{50} value, indicating the carboxylic acid was not optimally positioned to interact with PFTase Arg^{202}, the residue identified by mutational analysis to bind the C-terminal carboxylic acid in CaaX analogues [28]. Non-peptide N-phenylbenzamide PFTase inhibitors such as **12** are disclosed in a patent application from Merck, but specific biological data was not given [104]. A patent application from Biomeasure, Inc. covers imidazopiperazine PFTase inhibitors **13** and claims antiproliferative activity *vs.* a number of cell lines with mutated K-*ras* (A-427, Calu-1, MIA-PaCa) or wild-type K-*ras* (HT-29) [105].



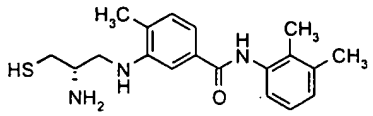
9
PFTase IC_{50} 5.5 nM



10a *meta* PFTase IC_{50} 250 nM
10b *para* PFTase IC_{50} 403 nM



11a *meta* PFTase IC_{50} 3800 nM
11b *para* PFTase IC_{50} 3150 nM



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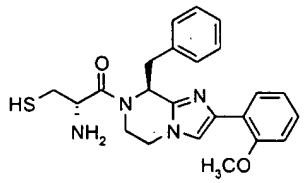

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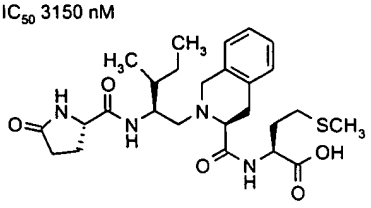

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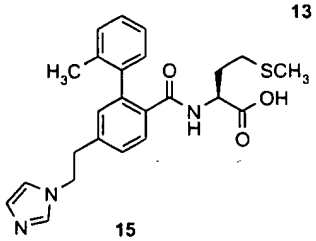

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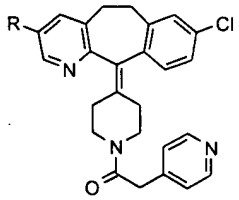
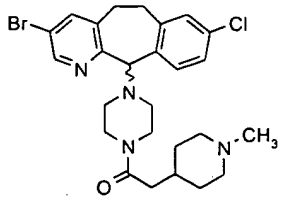


SCH 44342 R = H PFTase IC_{50} 250 nM
SCH 56580 R = CH_3 PFTase IC_{50} 40 nM



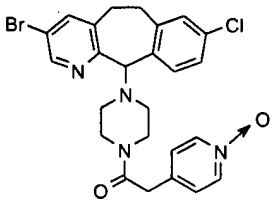
SCH 59425
PFTase IC_{50} 120 nM

2.2 Inhibitors without a thiol group

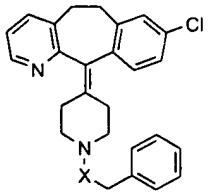
Concern about possible long-term adverse effects associated with a thiol dictates the absence of this functional group from a PFTase inhibitor intended for chronic administration. CaaX analogues with either pyroglutamate **14** [106] or imidazole **15** [107] in place of the usual N-terminal cysteine residue are the subjects of two patent applications. Inhibition potency was not stated.

While a number of cysteinyl replacements have appeared in the patent literature for CaaX analogues, PFTase inhibitors which are non-peptidic offer the best chance of obtaining a drug suited to oral administration. It is of no surprise then that pharmaceutical companies with PFTase inhibitor programmes have been concentrating in recent years on obtaining a non-peptide, non-thiol inhibitor suitable for clinical studies.

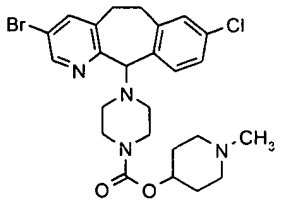
Several publications from Schering-Plough describe the synthesis and structure-activity studies of their previously reported non-peptide, non-thiol inhibitor **SCH 44342** [42,43]. **SCH 44342** competes with Ras binding to PFTase, and has an IC_{50} of 250 nM. In cell culture, it suppressed farnesylation in monkey kidney Cos cells with an IC_{50} of 1 μM. Although **SCH 44342** was 21% orally bioavailable in mice, its half-life was less than 10 min. Nonetheless, large oral doses of **SCH 44342** (100 mg/kg, bid) administered to nude mice with tumours from the human colorectal cell line SW-620 (containing a K-*ras* mutation) produced a 42% reduction in tumour weight after 35 days compared to vehicle control. The intrinsic potency of **SCH 44342** increased 6-fold by the addition of a methyl group at C-3 of the benzocycloheptapyridine ring. **SCH 56580** inhibited PFTase with an IC_{50} of 40 nM, however it remains equipotent to **SCH 44342** in cell culture. Related inhibitors include 3-bromobenzo-



SCH 59228
PFTase IC_{50} 47 nM



16a X = CO PFTase IC_{50} 0.8 μM
16b X = SO_2 PFTase IC_{50} 1 μM



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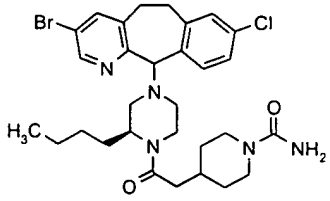

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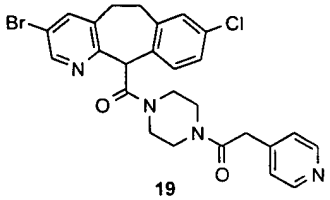

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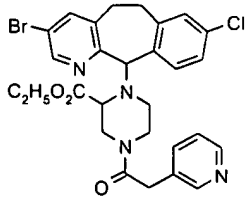


20

cycloheptapyridine analogues described in a meeting poster [44]. The 4-N-methylpiperidinylacetyl PFTase inhibitor **SCH 59425** (PFTase IC_{50} 120 nM) had 100% oral bioavailability and an 8.5 h half-life in mice, but had undesirable activity *vs.* muscarinic m_1 and m_2 receptors. The pyridylacetamide N-oxide **SCH 59228**, on the other hand, showed little m_1 and m_2 activity and was 38% orally bioavailable in cynomologous monkeys, with a half-life of 2.6 h. **SCH 59228** inhibited PFTase with an IC_{50} of 47 nM *in vitro*, 450 nM in Cos cells, and suppressed colony formation of H- and K-*ras* transformed NIH-3T3 cells with IC_{50} values of 1.2 and 4 μM, respectively. In studies in nude mice, **SCH 59228** given 50 mg/kg qid for 3 - 4 weeks showed 71 - 95% inhibition of H-*ras* tumour growth. The same protocol resulted in 31 - 42% inhibition of SW-620 tumour growth. Acetamides and sulfonamides such as **16a** and **16b** were essentially equipotent, although substitutions in the acetamide series which increased potency (such as 3- and 4-pyridylacetamide) were not reported in the sulfonamide series [45]. Six patent applications from Schering-Plough [108-113] and one from Pharmacopeia [114] cover 3-bromo-8-chloro-benzopyridocycloheptane inhibitors such as **17-20**, with the structurally related diphenylacetylpiperazine N-pyridylacetamide **21** the subject of a separate Schering-Plough patent application [115]. Specific biological data was not mentioned. Clinical trials with an orally bioavailable inhibitor, **SCH 66336**, are underway; details of the compound are not known at this time [15].

3. Farnesyl pyrophosphate analogues and antagonists

Researchers at Parke-Davis have further elucidated the biological properties of pentapeptide **PD083176**, discovered by high volume screening of peptide libraries [46]. **PD083176** is a 20 nM inhibitor of PFTase, and is competitive with the FPP substrate. Although the peptide structure may make **PD083176** look more like a Ras protein competitive inhibitor, it is less surprising that the pentapeptide is competitive with the lipophilic FPP substrate when its many hydrophobic substituents are considered. Unable to suppress farnesylation in cell culture, the most reasonable explanation is poor membrane penetration since microinjection of **PD083176** into Xenopus oocytes inhibited insulin-induced *ras*-dependent

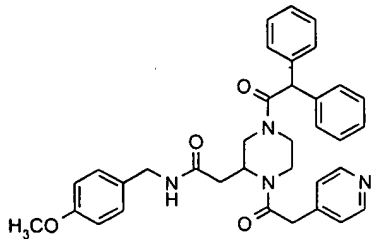
Table 1: IC_{50} dependence on phosphate ion concentration.

	IC_{50} (nM)			
$[PO_4^{2-}]$	PD083176	22	23	24
0	1950	510	7.5	3400
5 mM	165	100	6.8	415
30 mM	20	40	ND	145

ND: Not determined.

maturation. Extensive SAR studies on **PD083176** revealed that substitution of Phe for Tyr(OBn) or L-Ala for D-Ala gave an approximate 2-fold increase in potency [46]. Curiously, *in vitro* activity of PD083176 was dependent upon the concentration of phosphate anion in the assay mixture (**Table 1**) [47]. Both **PD083176** and the phenol **22** were more active under conditions in which phosphate anion was present than in phosphate-free conditions. It was speculated that anions such as phosphate bind at the enzyme active site and interact with **PD083176** to mimic an enzyme-product complex, which is consistent with the lack of phosphate-dependent activity of O-phosphotyrosine analogue **23**. The activity of truncated analogue **24** also showed a dependence on phosphate anion concentration; in the absence of phosphate, the IC_{50} was 3.4 μM, while in 30 mM phosphate buffer the IC_{50} dropped to 145 nM [48]. Compound **24** inhibited farnesylation in H-*ras* transfected NIH-3T3 cells with an IC_{50} of 1 μM, which appears to be most consistent with the *in vitro* IC_{50} determined in the presence of phosphate. Under assay conditions where **24** had an IC_{50} of 320 nM (5 mM phosphate buffer), the 2,2-dimethyl phenethyl amide **25** was 100-fold more potent [49,116]. Most notably, **25** inhibited colony formation of H-*ras* transformed cells with an IC_{50} of 180 nM, and blocked 88% of H-Ras tumour growth in nude mice when administered intraperitoneally at 150 mg/kg/day. Another patent application disclosed that histidine amide **26** had an IC_{50} of 130 nM *vs.* PFTase, and inhibits Ras farnesylation in cell culture with an IC_{50} of 2.5 μM [117].

Scientists at Istituto di Richerche di Biologia Molecolare designed a synthetic tetrapeptide combinatorial library to screen for novel FPTase inhibitors which did not have a thiol functional group [50]. The most active peptide, D-tryptophan-D-methionine-D-4-chlorophenylalanine-L-γ-carboxyglutamic acid **27** was also



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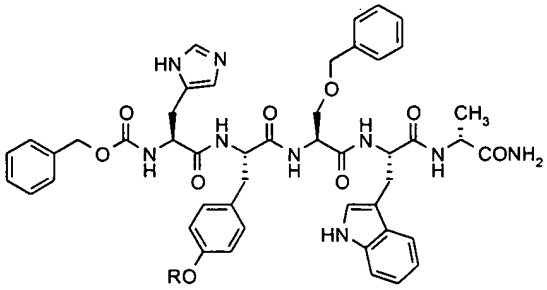
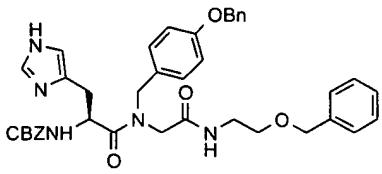


PD 083176 R = benzyl
22 R = H
23 R = PO_3^{2-}



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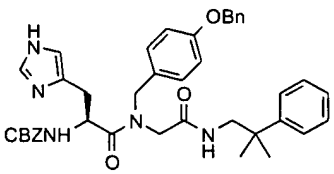
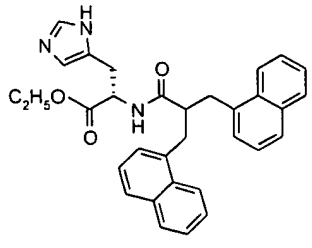


25
PFTase IC_{50} 4 nM



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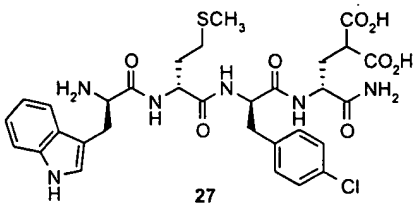
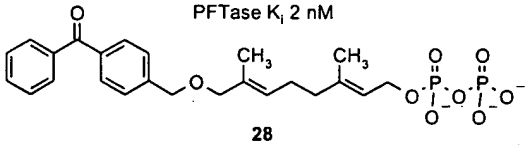
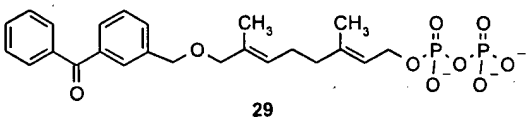


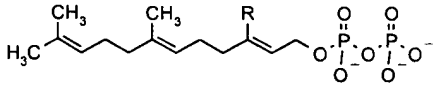
27
PFTase IC_{50} 42 nM
PFTase K_i 2 nM



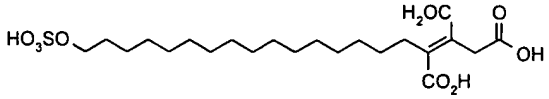
28
PFTase IC_{50} 1600 nM



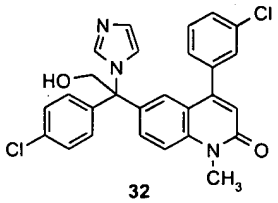
29
PFTase IC_{50} 850 nM



30a R = *c*-C_3H_5 PFTase K_i 500 nM
30b R = *t*-C_4H_9 PFTase K_i 310 nM



Oreganic acid
PFTase IC_{50} 14 nM



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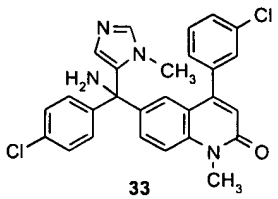

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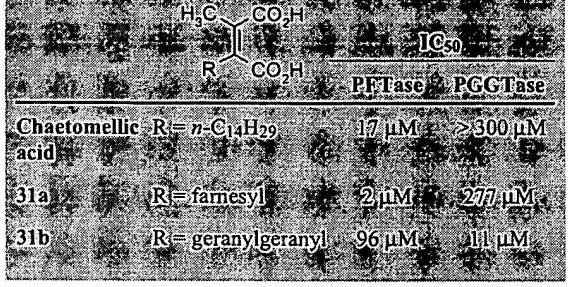
Table 2: Activity and selectivity of chaetomellic acid analogues *vs.* yeast enzymes.



		IC_{50}	
	R	PFTase	PGGTase
Chaetomellic acid	R = n-$C_{14}H_{29}$	17 µM	>300 µM
31a	R = farnesyl	2 µM	277 µM
31b	R = geranylgeranyl	96 µM	111 µM

competitive with respect to the FPP substrate, with a K_i of 2 nM. The tetrapeptide selectively inhibited PFTase over PGGTase I (IC_{50} 42 nM *vs.* > 50,000 nM).

Several publications describe the synthesis of benzophenone-containing analogues of FPP to use in photoaffinity labelling studies of yeast PFTase. Irradiation of yeast PFTase for 12 h in the presence of either isomer **28** (IC_{50} 1600 nM) or **29** (IC_{50} 850 nM) resulted in a 40% reduction in activity [51]. Compounds related to **28** and **29** were previously shown to label the β-subunit of yeast PFTase [52,53].

Building on earlier work [54,55], the FPP analogue 3-cyclopropyl-3-desmethylfarnesyl diphosphate **30a** was designed to be a mechanism-based inhibitor of PFTase [56]. It was envisioned that ionisation of the phosphate group in the enzyme active site to give the cyclopropylcarbinyl cation would result in alkylation and irreversible inhibition of the enzyme. Instead, however, **30a** effectively competed with FPP as an alternative substrate, with a K_i of 500 nM. The more sterically demanding 3-t-butyl-3-desmethylfarnesyl diphosphate **30b** had a similar K_i but was turned over exceptionally slowly by the enzyme. Time dependent inhibition of PFTase was not observed with either analogue.

A facile, two-step synthesis of the *bis*-carboxylic acid natural product chaetomellic acid A [57,58] and related analogues has appeared [59]. The farnesyl analogue **31a** (IC_{50} 2 µM) was more potent *vs.* yeast PFTase than chaetomellic acid (IC_{50} 17 µM) (**Table 2**). (Although these compounds were assayed for inhibition *vs.* yeast PFTase, note that chaetomellic acid is 300-fold more active *vs.* mammalian PFTase than yeast PFTase). Both chaetomellic acid and **31a** selectively inhibited PFTase over PGGTase I, however this selectivity was reversed for the geranylgeranylated

analogue **31b** (PGGTase IC_{50} 11 μM, PFTase IC_{50} 96 μM).

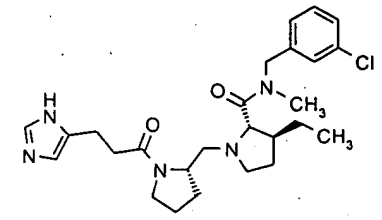
A sulfated tricarboxylic acid inhibitor of PFTase, oreganic acid, was isolated from fungal extracts [60,61]. It had an IC_{50} of 14 nM *vs.* PFTase and 60 μM *vs.* PGGTase and was competitive with the FPP substrate. Removal of the sulfate group or esterification of the carboxylic acids resulted in 100- to 1000-fold loss in potency.

4. PFTase inhibitors with an undefined enzyme inhibitory mechanism

A number of interesting compounds have appeared in recent patent publications. Although biological data generally are not disclosed, many of the chemical structures lack both mercaptan and carboxylic acid groups and thus appear to be well suited to drug development programmes.

Patent applications from Janssen disclose two series of imidazolylmethyl quinolinone PFTase inhibitors **32** and **33** [118,119]. The only specifically claimed compounds were the two stereoisomers of **33**, one of which (the (+) isomer **R115777**) inhibited tumour growth of a cell line in nude mice when dosed at 25 mg/kg p.o. qid. Janssen is in clinical trials with **R115777**, which is being given by oral administration; **R115777** inhibits PFTase activity with an IC_{50} of 7.9 nM and 40% of PGGTase activity at 50 μM [13]. Initial studies indicate the compound is bioavailable in man, with a mean terminal half-life of 13 - 14 h. From the initial dose of 25 mg p.o. bid through 5 dose escalations, **R115777** showed acceptable toxicity, allowing further dose increases [14].

Additional imidazole-containing inhibitors were disclosed by Merck in patent applications. The imidazolylproprionamide *bis* pyrrolidine **34** and the N-imidazolylmethyl N-*p*-nitrobenzylamine **35** were claimed as PFTase inhibitors [120,121]. A series of patents have published claiming *p*-nitrobenzylimidazole or *p*-cyanobenzylimidazole attached to a number of different heterocycles, including piperazines related to **36** [122-126], piperidines similar to **37** [127-129], imidazolidinone **38** [130], biaryl and heterobiaryl compounds like **39** and **40** [131-138], benzodiazepinone **41** [139], and acyclic compounds related to **42** and **43** [140-147]. Specific biological data was not included in these patents. Imidazolylmethyl benzodiazepine **44** appears in a patent application



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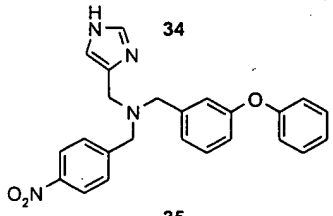

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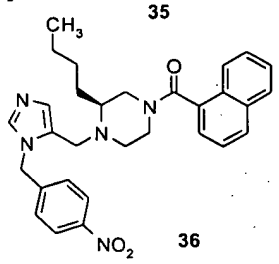

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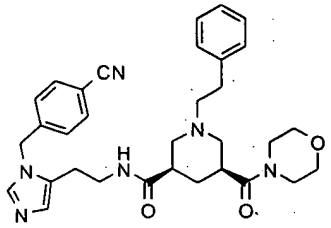

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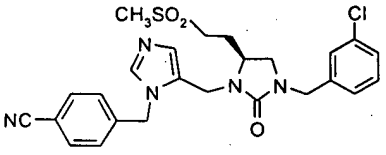

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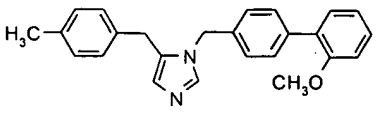

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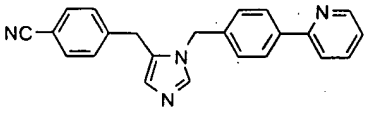

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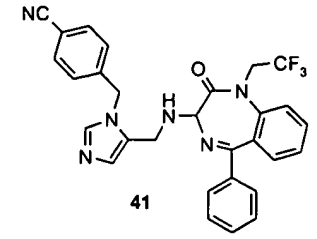

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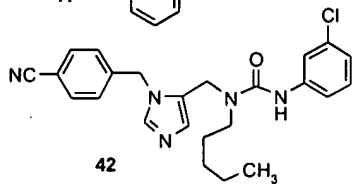

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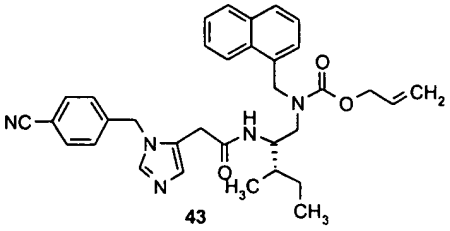

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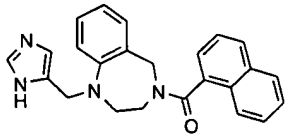

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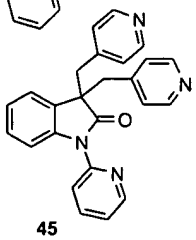

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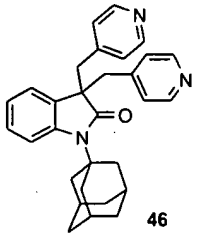

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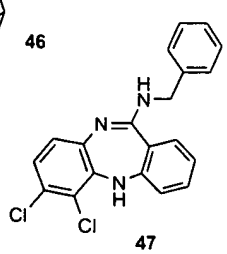
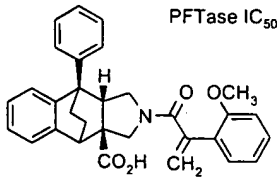


47

PFTase IC_{50} 800 nM



48

PFTase IC_{50} 50 nM

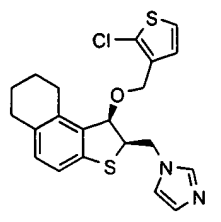
from Bristol-Myers Squibb, representing another class of imidazole-containing PFTase inhibitors [148].

Other interesting compounds published in the patent literature do not have an imidazole, but instead contain other nitrogenous heterocycles like pyridine. Patent applications from Pfizer disclose pyridylmethyl-substituted indolinones **45** [149] and **46** [150] as PFTase inhibitors. No biological data are given. The pyridyl-containing tricyclic dibenzodiazepine **47** (IC_{50} 800 nM) is one of several specifically claimed PFTase inhibitors in a patent application from Warner-Lambert [151]. Tetracyclic benzo[f]isoindole compounds such as **48** (IC_{50} 50 nM) comprise a patent application from Rhône-Poulenc Rorer [152].

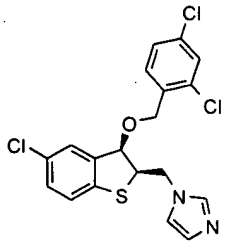
A pharmacophore model correlating the activity of benzocycloheptapyridine PFTase inhibitors related to **SCH 44342** was generated by three-dimensional quantitative structure-activity relationship (3D-QSAR) computational analysis [62]. Using this hypothesis, three-dimensional search of the Schering-Plough database identified 718 hits. Biological testing ultimately identified imidazolylmethyl dihydrobenzothiophenes **49** (IC_{50} 200 nM) and **50** (IC_{50} 200 nM) as novel PFTase inhibitors. Despite their similar structures, **49** was only 3.5-fold selective for PFTase over PGGTase inhibition, while **50** was 84-fold selective. Other data, such as kinetic mechanism and cell potency, were not discussed.

A number of patent applications have appeared claiming di- and tricarboxylic acid PFTase inhibitors. Triaryl carboxylic acids **51-53** are disclosed in Banyu patent applications as having IC_{50} values of ≤ 0.1 nM [153-160]. Given its highly polar nature, it is somewhat surprising that tricarboxylic acid **51** displayed activity in cell culture, where it inhibited Ras processing with an IC_{50} of 3.5 μM. Antitumour activity of **51** in a mouse tumour model was also observed. Cyclobutane di- and tricarboxylic acid PFTase inhibitors from Abbott (**54**) continue to appear in the patent literature [161,162]. Abbott has also claimed substituted pyromellitic acid **55** as a PFTase inhibitor which blocks Ras farnesylation by 98% at 1 μM [163].

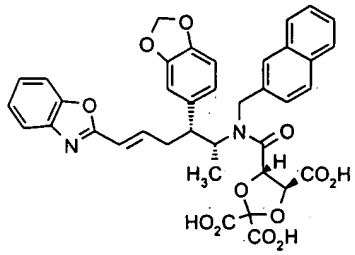
Kurasoins A and B are α-hydroxyketones isolated from fermentation broths of a *Paecilomyces* sp. soil fungus, which inhibit PFTase with IC_{50} values of 59 μM [63-65]. Additional members of the cylindrol and ascochlorin families of natural products (families associated with PFTase inhibitory activity) have been isolated from *Cylindrocarpon lucindum* and their PFTase activity characterised [66]. Compound **56** was



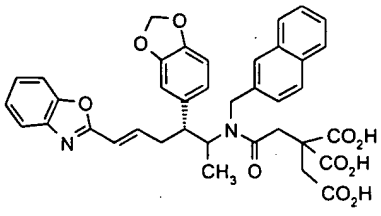
49
PFTase IC_{50} 200 nM



50
PFTase IC_{50} 200 nM



51
PFTase IC_{50} 0.1 nM



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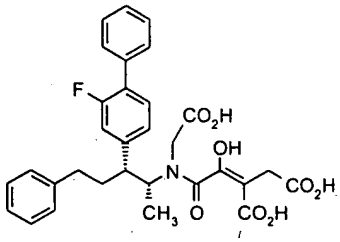
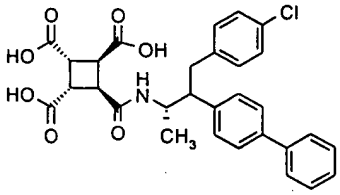
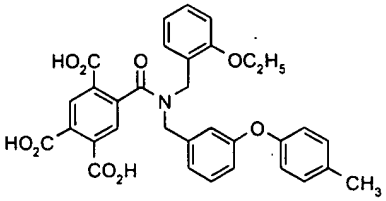


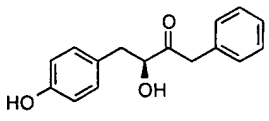
53
PFTase IC_{50} 0.1 nM



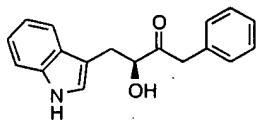
54
81% inh PFTase @ 150 nM



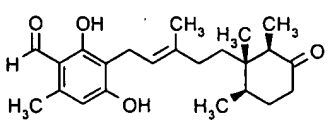
55
98% inh PFTase at 1 μM



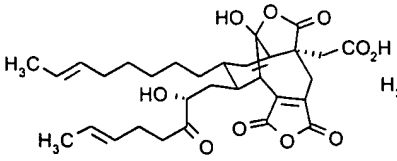
Kurasoin A
PFTase IC_{50} 59 μM



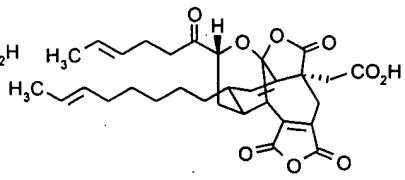
Kurasoin B
PFTase IC_{50} 59 μM



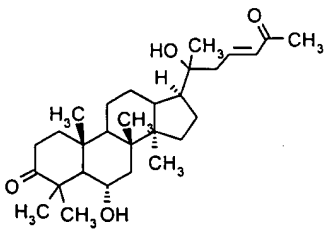
56
PFTase IC_{50} 430 nM



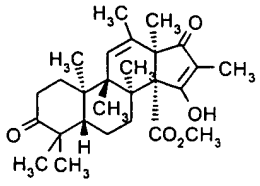
CP-225,917
PFTase IC_{50} 6 μM



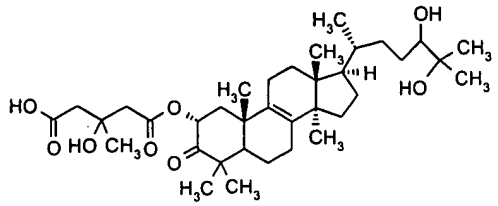
CP-263,114
PFTase IC_{50} 20 μM



Rhombenone
PFTase IC_{50} 2.3 μM



Andrastin D
PFTase IC_{50} 26 μM



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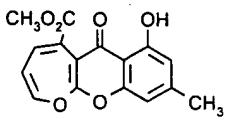

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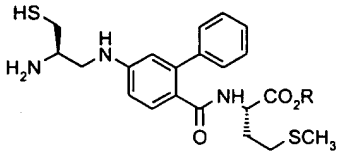
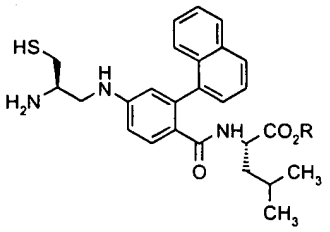


FTI-276 R = H PFTase IC_{50} 0.6 nM

FTI-277 R = CH_3



GGTI-297 R = H PGGTase IC_{50} 54 nM

GGTI-298 R = CH_3

the most potent inhibitor isolated, with an IC_{50} of 430 nM *vs.* hr-PFTase; a more detailed kinetic analysis showed that, like Cylindrol A, compound **56** was non-competitive with respect to both Ras peptide and FPP substrates. A publication more fully describes the fungal metabolites **CP-225,917** and **CP-263,114**, the subjects of an earlier patent application [67]. These compounds inhibit PFTase with IC_{50} values of 6 and 20 μM, respectively, but are only 7-fold selective for inhibition of PFTase over squalene synthase. A triterpene of the dammarane family, rhombenone, was isolated from the leaves of the medicinal plant *Hedera rhombea* bean, and was found to inhibit PFTase with an IC_{50} of 2.3 μM [68]. A fourth member of the andrastin family of *penicillium* metabolites, andrastin D, was isolated and characterised [69]. Andrastin D, similar to andrastins A-C, inhibited PFTase with an IC_{50} of 26 μM. The steroid **57** [164] and tricyclic **58** [165] were claimed in a patent application from Merck as inhibitors of PFTase, although no biological data were given.

5. Conclusion

Activity both in the patent and scientific literature shows that interest in PFTase inhibitors remains high. Most of the current effort is focused on obtaining non-thiol PFTase inhibitors that can be administered orally, in anticipation that it will be necessary to maintain PFTase inhibition over extended periods of time. The recent publication of the x-ray crystal structure of rat PFTase should ultimately result in the design of novel PFTase inhibitors. As the first compounds move through clinical trials, basic research continues to elucidate fundamental aspects of protein prenylation and the biological consequences of suppressing protein prenylation.

6. Expert opinion

Results from clinical trials of the first generation of PFTase inhibitors will certainly inform and direct future research in the area. Important considerations include the choice of clinical markers to follow patient response, as well as clinical end-points to judge the success or failure of therapy. Combination therapy with other cancer drugs and a PFTase inhibitor will certainly be addressed. Coincident suppression of farnesylation and radiation therapy may impart added benefit, since **FTI-277** is reported to be a radiation sensitiser [70].

Given the differential binding of K-Ras to microtubules discussed earlier, it is reasonable to expect other physiological distinctions between H-, K-, and N-Ras proteins will appear. The effects of PFTase substrates other than Ras on cell growth and differentiation will also influence the direction of future research.

Additional biochemical studies need to define the role that protein geranylgeranylation plays in intracellular processes. Researchers at the University of Pittsburgh found that blocking PGGTase activity with the PGGTase inhibitor prodrug **GGTI-298** in either fibroblasts or human epithelial tumour cell lines affects cell cycle progression by stably arresting cells

in G_0/G_1 [71-73]. It was also found that **GGTI-298** increased the expression of $p21^{WAF}$ independent of p53; $p21^{WAF}$ is a cyclin-dependent kinase inhibitor normally up-regulated by the p53 tumour suppressor protein in response to DNA damage. In contrast, the PFTase inhibitor prodrug **FTI-277** had either varying or no effect on cell cycle distribution, and did not induce the expression of $p21^{WAF}$. Overall, these results suggest that a PGGTase inhibitor might be effective at inducing growth arrest in cells lacking functional p53. The relative influence of GGTase-I and PFTase inhibition in these experiments is not clear, however, since the active forms of **GGTI-298** (**GGTI-297**) and **FTI-277** (**FTI-276**) are equipotent GGTase-I inhibitors *in vitro* (IC_{50} values 54 nM and 53 nM, respectively), differing only in their activity *vs.* PFTase (IC_{50} values 190 nM and 0.6 nM, respectively) [71]. Clearly, PGGTase and PFTase inhibitors with greater selectivities are needed to elaborate on these results.

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